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ORIGINAL PAPER

Antibiotics ingestion altered the composition of gut microbes and affected the development and reproduction of the fall armyworm

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Abstract

A dynamic homeostasis between gut microbiome and the host is essential for animals. Antibiotics feeding may be a good way to study the function of microbes in insects due to efficiency and a linkage with pest control. Here, by using 16S rDNA sequencing, we show antibiotics feeding significantly altered the composition and diversity of microbes in different stages of *Spodoptera frugiperda* and showed dose dependent effects. Antibiotics ingestion resulted in a dramatic reduction of *Enterococcus* in larvae and *Klebsiella* in adults, but increase of *Weissella* in larvae and *Pseudomonas* in pupae and adults. *Enterococcus* spp in the lepidopteran gut may play a protective role against insect pathogens and *Klebsiella* spp may have positive effects on insect fecundity. Some strains from *Pseudomonas* and *Weissella* are pathogens or opportunistic pathogens. Further biological assay showed that antibiotics treatment significantly affected the fitness of treated insects and their untreated offspring, with treated insects and their offspring having longer developmental period but lower body weight, survival rate, flight capacity and fecundity than those of controls. Lepidopterans may rely on gut microbiome for some digestions and previous study indicated that antibiotics-induced dysbiosis of gut microbes affects many biological processes of *S. frugiperda*. Therefore, it is possible that antibiotics disrupted the homeostasis of gut microbes and the host, which then negatively affected the survival and reproduction of *S. frugiperda*. These findings contribute to a better understanding of the role of the microbiota in insects and will aid in the development of environmentally friendly management techniques for this pest.

Keywords *Spodoptera frugiperda* · Gut microbiome · Development · Reproduction · Antibiotics · Dysbiosis

Introduction

The fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is currently a major worldwide agricultural pest. This moth pest is native to tropical and subtropical regions in the Americas (Kenis et al. 2023) and before 2015 there was no report on its distribution outside the Americas. It was first recorded in the southwest of China at the end of 2018, and then spread to the vast areas of China soon after (Jiang et al. 2019). This pest can cause substantial economic losses to corn production. In China, an estimation by Qin et al. (2020) suggested that the potential annually economic loss on corn by this pest ranged from $17,286 m to $52,143 m. Moreover, this pest is also notorious for its long-distance migration ability (Westbrook et al. 2016), strong pesticide resistance (Li et al. 2019) and high fecundity (Kenis et al. 2023). Currently, broad-spectrum chemical insecticides are primarily used to control *S. frugiperda*, which has further promoted its resistance to conventional insecticides, and even Bt toxins (Li et al. 2019; Overton et al. 2021). Environmental friendly and sustainable management strategies are thus required for the better control of this pest in the future.

Animals, including insects, depend on their gut microbiomes for survival. The contribution of gut microbes to

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host nutrition occur in diverse ways, such as by providing digestive enzymes and nutritional components (Dillon and Dillon 2004). In phloem-feeding insects, amino acid and vitamin-supplementing symbionts have evolved to compensate the limited dietary nitrogen in the diet (Dillon and Dillon 2004; Voirol et al. 2018). Previous studies, particularly on termites and cockroaches, have greatly revealed the contribution of gut bacteria to nutrition for hosts living on plants and other suboptimal diet (Bar-Shmuel et al. 2020). A study on the diamondback moth showed that many isolated gut bacteria could fix nitrogen in vitro (Indiragandhi et al. 2008). Plant cell wall degrading enzymes, including cellulases, hemicellulases and pectinases, are responsible for the break-up of plant cell walls to provide carbohydrates to insects (Watanabe and Tokuda 2010). However, most of the lepidopterans studied, lack such cellulase-encoding genes (Voirol et al. 2018). Xia et al. (2017) identified thousands of genes from the gut microbiome of the diamondback moth that encode cellulases and carbohydrate-active enzymes. These findings imply that lepidopteran insects rely on symbionts for cellulose digestion.

In addition to nutrient acquisition, resident gut bacteria provide protection against pathogenic colonization of the gut (Florez et al. 2015). For example, in the oriental tea tortrix, aseptically-reared caterpillars were more susceptible to *Bacillus thuringiensis* than normally-reared ones (Takatsuka and Kunimi 2000). Moreover, gut bacteria have also been shown to benefit herbivores by counteracting plant toxic defenses (van den Bosch and Welte 2017). For example, *Rhodococcus* spp. in the gut of the gypsy moth can degrade monoterpenes (van der Vlugt-Bergmans and van der Werf 2001), which allow this moth to tolerate diets enriched with monoterpenes (Broderick et al. 2004).

Studies have also demonstrated that microorganisms can have beneficial or detrimental influence on the reproductive function and fitness of males and females (Rowe et al. 2021). For instance, a study by Otti et al. (2013) showed that exposing the bedbug to polymicrobial mixture (such as *Acinetobacter*, *Alcaligenes*, *Bacillus*, and *Staphylococcus*) significantly increased sperm mortality (up to 40%). Some *Enterococcus* species, such as *E. faecalis*, showed negative impact on the fecundity of fruit flies (Akami et al. 2019; Noman et al. 2021). While other bacterial species, such as *Klebsiella pneumonia*, *Citrobacter braakii*, *Pantoea dispersa*, and *Enterobacter cloacae*, had positive effects on the fecundity of fruit flies (Akami et al. 2019; Rashid et al. 2018). Further, the symbiotic bacterium, *Wolbachia*, has been shown to play roles in the parthenogenesis of insects (Ma and Schwander 2017) and the provisioning of riboflavin (Moriyama et al. 2015). However, evidence of how symbionts influence reproduction and the underlying mechanisms remain poorly understood.

In addition to the use of germ-free insects (such as above-mentioned aseptically reared insects), suppressing resident microorganisms using antibiotic treatments can effectively eliminate bacteria or disrupt the balance of insect gut microbiota, which enables their functions to be evaluated (Lee et al. 2017; Noman et al. 2021). For example, the brown planthopper, *Nilaparvata lugens*, devoid of its symbionts, showed increased susceptibility to antibiotics treatment (Tang et al. 2021). In the pumpkin fruit fly, larvae feeding on antibiotics resulted in marked changes in bacterial diversity and effect on ovary development (Noman et al. 2021). Therefore, modifying symbiotic microbes can be a potential management strategy for the control of agricultural insect pests (Perilla-Henao and Casteel 2016; Beck and Vannette 2017).

A number of studies have been conducted on the gut microbial community in *S. frugiperda* populations from different hosts and different regions (e.g., Gichuhi et al. 2020; Zhang et al. 2022) and their modulating effect on plant defense responses (Acevedo et al. 2017). Further, dysbiosis of gut microbiota by antibiotics exposure was shown to have affected energy and metabolic homeostasis in S. *frugiperda* (Chen et al. 2021). In the present study, we further studied the composition and diversity of bacteria in different life stages (larvae, male and female pupae and adults) of *S. frugiperda* under both normal and antibiotic-treated conditions using 16S rDNA sequencing. We also evaluated the effect of antibiotics treatment on the fitness of *S. frugiperda* in the treated generation and their untreated offspring. We discuss the possible links between gut bacteria and the survival and reproduction of *S. frugiperda*.

Materials and methods

Insects

Spodoptera frugiperda larvae were collected in a corn field near Dongchuan town in Yunnan Province, China. The larvae were then reared on artificial diet (Wu et al. 2023) under 28 ± 1 °C and 60–80% relative humidity with 14:10 h light:dark photoperiod. Adults were fed with a 10% honey solution. Their offspring was used for the present study. Under this rearing condition, the life cycle of *S. frugiperda* was about 3 d for eggs, 16 d for larvae, 8 d for pupae and 10 d for adults.

Antibiotics treatment

Four antibiotics (ampicillin, streptomycin, tetracycline and metronidazole) with specific concentrations were selected for this experiment according to previous studies (Noman et al. 2021; Bai et al. 2019). Two antibiotic feeding

treatments (T1 and T2) and one control (CK) were set up for *S. frugiperda*: T1: larvae were fed with artificial diet to which the four antibiotics had been independently added, at a concentration of 1 mg of each antibiotic per 1 g diet. Larvae fed from the hatching to pupation period. Adults were fed with 10% honey solution to which the four antibiotics had been independently added, at a concentration of 1 mg of each antibiotic per 1 ml solution. T2: larvae were fed with artificial diet to which the four antibiotics had been independently added, for 8 days since hatching, and then fed with diet without antibiotics. Adults were fed with 10% honey without antibiotics. CK: larvae were fed with artificial diet without antibiotics, and adults were fed with 10% honey solution without antibiotics.

Effect of antibiotics feeding on the composition of bacteria in different life stages

Following the above feeding treatments, insects were sampled at different stages to study the composition of gut bacteria: (1) matured larvae (which had stopped feeding and entering pupation) were sampled from each treatment and named accordingly as T1L, T2L and CKL; (2) 4-d-old male and female pupae were sampled from each treatment and named accordingly as T1PM, T2PM and CKPM for male pupae, and T1PF, T2PF and CKPF for female pupae; and (3) 4-d-old virgin male and female adults were sampled from each treatment and named accordingly as T1AM, T2AM and CKAM for male adults, and T1AF, T2AF and CKAF for female adults. Three replicates were used for each sample and eight insects were used in each replicate. Before sampling, the insects were rinsed twice with sterile water and were surface-sterilized in 75% ethanol for 90 s, and then rinsed twice again using sterile water. The whole body of larvae and pupae, and the abdomen of adults (cut from the sterilized adults using sterile scissors) were sampled and stored at − 80 °C until use.

Total genome DNA was extracted from samples using the CTAB/SDS method. DNA purity and concentration was examined by 1.0% agarose gel electrophoresis. DNA was then diluted to 1 ng/μL using sterile water and was submitted for 16S rDNA gene sequencing using the Illumina NovaSeq PE250 platform (Novogene Bioinformatics Technology Co., Ltd., Beijing, China). The obtained raw data were deposited into the NCBI Sequence Read Archive (SRA) database (Accession No.: PRJNA803874).

Clean reads were obtained by removing chimera sequences and low-quality reads from the raw reads. Uparse (Version 7.0.1001) (Edgar 2013) was used for subsequent sequence analysis. Sequences with ≥ 97% similarity were assigned to the same OTUs. Representative sequence for each OTU was screened for further annotation using the Silva Database (Wang et al. 2007) based on the Mothur algorithm.

QIIME (Version 1.7.0) was used for the analysis of alpha diversity to reveal the complexity of species within samples. Beta diversity to assess the differences in microbial community between groups was also determined. The significance of differences between groups were tested using non-parametric multivariate analysis of variance (NPMANOVA) based on the Bray–Curtis metrics, and then visualized accordingly using Principal coordinates analysis (PCoA) based on the Bray–Curtis metrics. NPMANOVA was performed using the vegan and phyloseq packages in R (Version 4.0.3). Linear discriminant analysis (LDA) of effect size was used to determine OTUs that discriminated among the populations with an LDA score more than 4.0.

Effect of antibiotics feeding on development, flight capacity and reproduction

Following the above feeding treatments, the duration of larval and pupal stages, the body weight of mature larvae and newly-emerged pupae and adults, pupation rate (number of pupae/number of start larvae%), eclosion rate (number of adults/number of pupae%) and survival rate from larvae to adults (number of adults/number of start larvae%) were recorded. The pupation rate, eclosion rate and survival rate were determined with three replicates per each treatment, with 120 larvae per replicate. The developmental duration was determined using one insect as a replicate, with 168 larvae and 200 (100 males and 100 females) pupae randomly selected from each treatment for the measurements. The body weight at different stages was determined using one insect as a replicate, with 120 larvae, 200 (100 males and 100 females) pupae and 120 (60 males and 60 females) adults randomly selected from each treatment for the measurements.

Three-day old virgin male and female adults from each treatment were used for flight capacity tests using a computer-monitored flight mill (Jiaduo Industry & Trade Co., Ltd., Hebi, China) following the method outlined by Guo et al. (2023). For each test, a moth was anesthetized by CO_2 and adhered to the tip of the mill cantilever via the moth's pronotum using Supertite glue (Gymcol Adhesives Co., Ltd., Pinghu, China). Each test was conducted for 8 h during the scotophase under the same condition as above. The number and duration of flight mill revolutions were recorded and flight distance (km), flight duration (h) and flight speed (km/h) for each moth was computed using the mill supporting software. A moth was considered a replicate and 35 moths were used for each treatment (n = 35).

Three-day old virgin male and female adults from each treatment were collected and paired in plastic boxes (25 cm long, 15 cm wide and 8 cm high; one pair per box) for

mating and oviposition. Each box was provided a paper strip (15 × 20 cm) folded in zigzag fashion as an oviposition substratum and 10% honey solution as food. Eggs were collected and incubated in petri dishes (8.5 × 1.5 cm) under the above conditions. The number of hatched eggs (larvae) was recorded 4 days after incubation. Twenty pairs were used for each treatment (n = 20).

To exclude the negative effect of antibiotic treatment on insects, the offspring (named accordingly as CK-F1, T1-F1 and T2-F1) from the antibiotic-treated insects were reared on artificial diet and 10% honey solution without antibiotics, under the same rearing conditions. Their growth, flight capacity and reproduction were also measured accordingly as above.

Significant differences between treatments on data of development, flight capacity and reproduction were determined. A goodness-of-fit test was performed to test the data distribution. Percentage data were arcsin square root-transformed before the test. Data on flight capacity and reproductive fitness were analysed using a multivariate ANOVA (MANOVA) followed by Tukey's studentized range for multiple comparisons between treatments, as flight distance, flight duration, and flight speed were intercorrelated (Scheiner 2001), as well as number of eggs laid, larvae and egg-hatching rate (Xu and Wang 2009). Other normally distributed data was analysed using an ANOVA followed by Tukey's studentized range test for multiple comparisons. Non-normally distributed data even after transformation were analysed using the nonparametric Kruskal–Wallis (K-W) test followed by Dunn's procedure with Benjamini−Hochberg correction for multiple comparisons. Rejection level was set at $\alpha < 0.05$. Values reported here are means ± SE.

Results

Sequencing and quality control

Sequencing generated ~ 70,000 clean reads from each of the 45 sequenced libraries, with the average length being 411–428 bp (Table S1). The percentages of Q20 and Q30 of all samples' clean reads ranged from 97.41 to 98.40% and from 92.51 to 94.63%, respectively. These sequences were clustered into 3958 OTUs (Table S2). Rarefaction analysis showed a saturating number of OTUs (Fig. S1), which indicated an adequate sequencing output for all samples.

Diversity indices of bacterial OTUs

The Good's coverages of all samples were all greater than 99% (Table 1), which suggested that the number of clones sampled was sufficient to provide an adequate estimation of bacterial diversity in *S. frugiperda*. The number of OTUs in different samples ranged from 160 to 916 (Table 1); it was low in larvae and adults, but high in pupae for untreated or antibiotic-treated insects. Within the total samples, about 26.48% (1048/3958) OTUs were shared by different treatments (Fig. 1a) and within untreated insects (CK), about 5.04% (102/2025) OTUs were shared by males and females of different developmental stages (Fig. 1b). Accordingly, the alpha diversity indices, Shannon, Simpson and Chao1 showed the variation in bacterial diversity among different stages and between control and treatment groups (Table 1).

Beta diversity analysis based on Bray–Curtis distance (illustrated by PCoA) further showed significant variances in the composition of OTUs within different developmental

Table 1 Alpha diversity indices of bacteria in different samples of *S. frugiperda*

Sample name	Clean reads	Number of OTUs	Shannon	Simpson	Chao1	Good's coverage
CKL	63,214	224	1.295	0.281	286.651	0.998
CKPF	64,074	622	3.471	0.711	820.566	0.993
CKPM	62,140	659	4.011	0.704	895.146	0.993
CKAF	61,025	164	2.583	0.677	227.559	0.998
CKAM	62,145	244	2.68	0.656	340.002	0.997
T1L	65,981	280	4.224	0.849	330.004	0.998
T1PF	66,015	916	5.94	0.913	1077.537	0.993
T1PM	60,870	857	7.171	0.958	1023.465	0.995
T1AF	43,140	304	5.687	0.965	380.907	0.998
T1AM	60,385	359	5.171	0.928	403.518	0.998
T2L	65,233	195	1.637	0.425	253.466	0.998
T2PF	58,587	697	4.923	0.905	889.676	0.993
T2PM	62,425	654	5.23	0.925	872.827	0.993
T2AF	65,032	160	2.571	0.665	221.764	0.998
T2AM	64,234	197	3.002	0.775	265.306	0.998

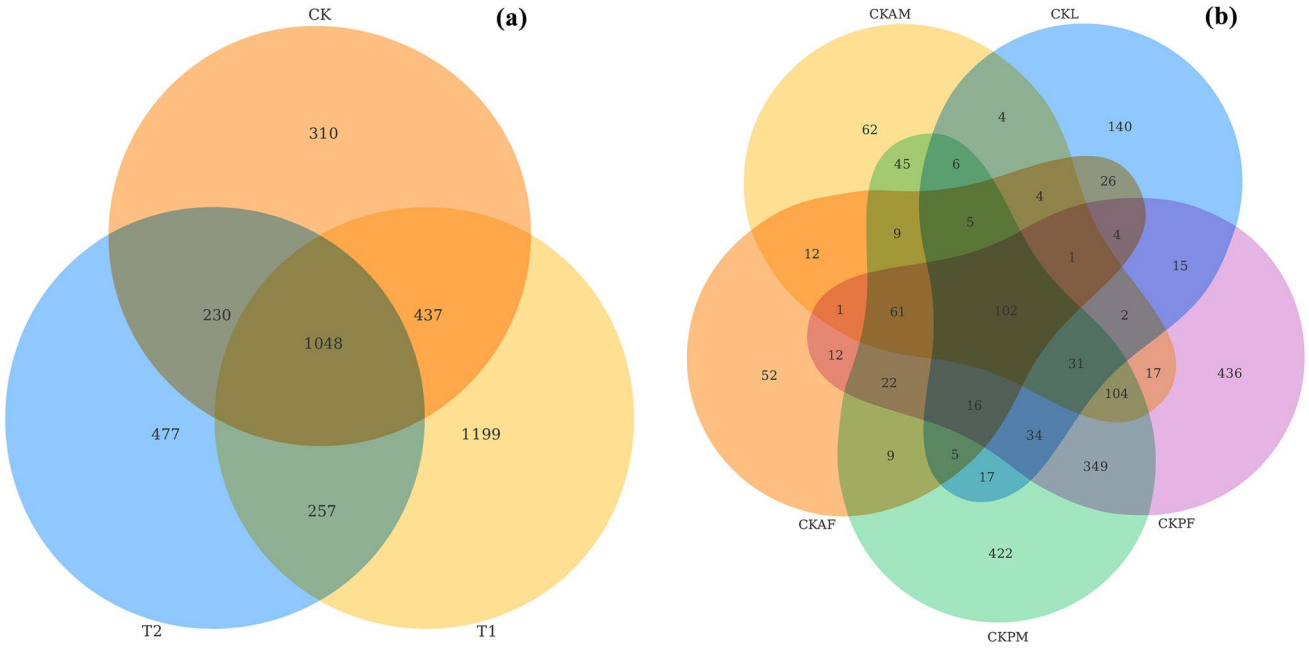


Fig. 1 The Venn diagram of OTUs. **a** The overlap of OTUs of different treatments; **b** The overlap of OTUs at different developmental stages of untreated *S. frugiperda*. The overlapping circles represent common OTUs among all combinations

stages of untreated insects (Fig. 2a), and between different treatments (Fig. 2b). Within untreated insects, pairwise comparisons (Table S3) showed that the differences between CKL and CKA and between CKP and CKA were significant ($P < 0.05$), while the difference between CKL and CKP was not significant ($P > 0.05$); also, no significant difference was found between males and females either in pupae or adults ($P > 0.05$). Pairwise comparisons between different treatments (Table S3) revealed that the differences between CK and T1 and between T1 and T2 were significant ($P < 0.05$),

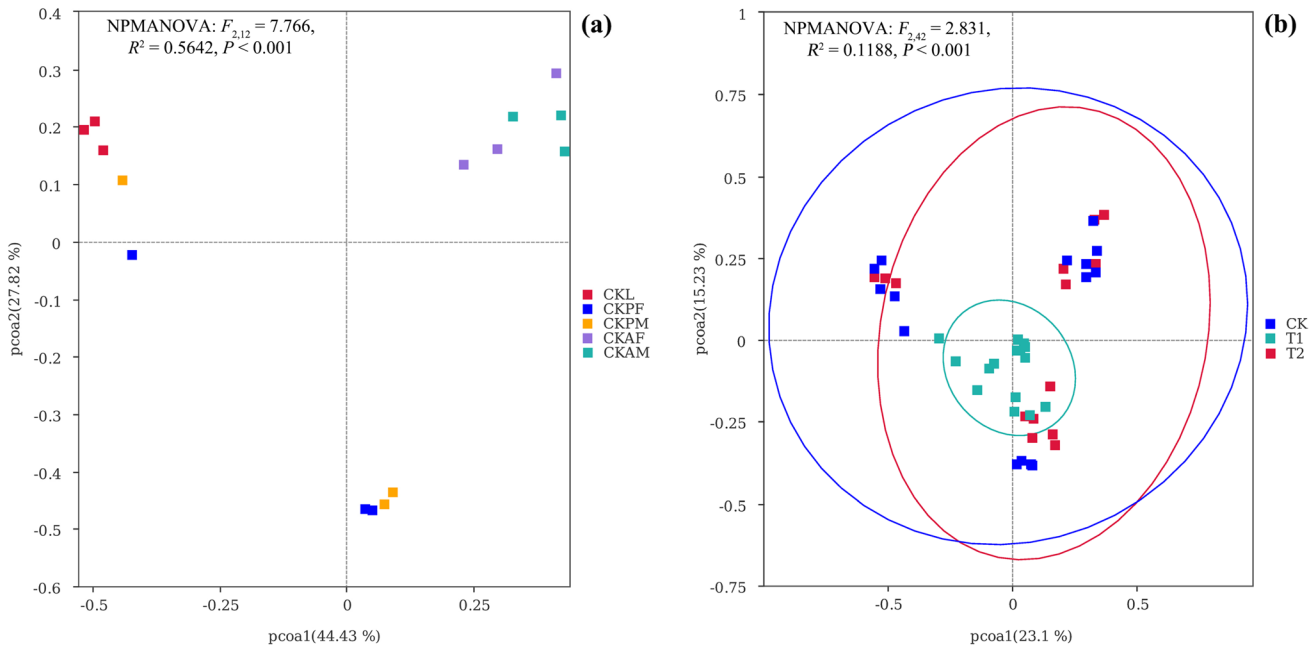


Fig. 2 PCoA ordination based on Bray–Curtis distances. **a** PCoA ordination at different developmental stages of untreated *S. frugiperda*; **b** PCoA ordination of different treatments

whereas the difference between CK and T2 was not significant ($P > 0.05$).

Taxonomy assignment

The 3958 OTUs obtained (Table S2) were classified into 42 phyla (Table S4; Fig. 3a), 87 classes (Table S5), 203 orders (Table S6), 309 families (Table S7), 583 genera (Table S8; Fig. 3b) and 370 species (Table S9). The abundance pattern at the phylum level showed that Firmicutes and Proteobacteria were the most predominant bacterial Phyla in *S. frugiperda* and showed obvious variance at different development stages and treatments (Fig. 3a; Fig. S2; Table S4). The abundance of Firmicutes was very high at the larval stage, but decreased with development (lower in pupae and even lower in adults), whereas the abundance of Proteobacteria showed an opposite change in trend (low in larvae and then increased with development) (Fig. 3a).

OTUs clustering at the genus level also revealed obvious variance at different developmental stages and treatments (Fig. 3b; Fig. S2; Table S8). At the larval stage, *Enterococcus* was the predominant bacterial genus in CKL (86.59%), which was relatively lower in T2L (77.86%), and much lower in T1L (7.20%); *Globicatella* was the dominant genus in T1L (29.43%). At the pupal stage, *Enterococcus* was still the predominant bacterial genus in control females (CKPF, 29.84%) and males (CKPM, 31.74%). *Acetobacter* and *Staphylococcus* also showed high diversity (11.74–24.02%) in these insects. *Enterococcus* also was the most dominant genus in T1 female pupae (T1PF, 24.90%) and male pupae (T1PM, 4.35%), with *Pseudomonas* being the second dominant genus (5.88 for T1PF and 2.58 for T1PM). *Enterococcus*, *Pseudomonas*, *Weissella*, *Acetobacter* and *Staphylococcus* were the dominant genera in T2 female and male pupae (6.95–17.79%). At the adult stage, the dominant genera in control females were *Pseudomonas* (34.91%), *Klebsiella* (23.42%) and *Enterococcus* (19.07%), while in control males were *Klebsiella* (48.92%) and *Weissella* (16.99%). *Staphylococcus* was the most dominant genus in T1 females (7.40%) and males (19.40%). *Gluconobacter* (24.89%), *Pseudomonas* (22.99%), *Klebsiella* (18.09%) and *Enterococcus* (14.08%) were the dominant

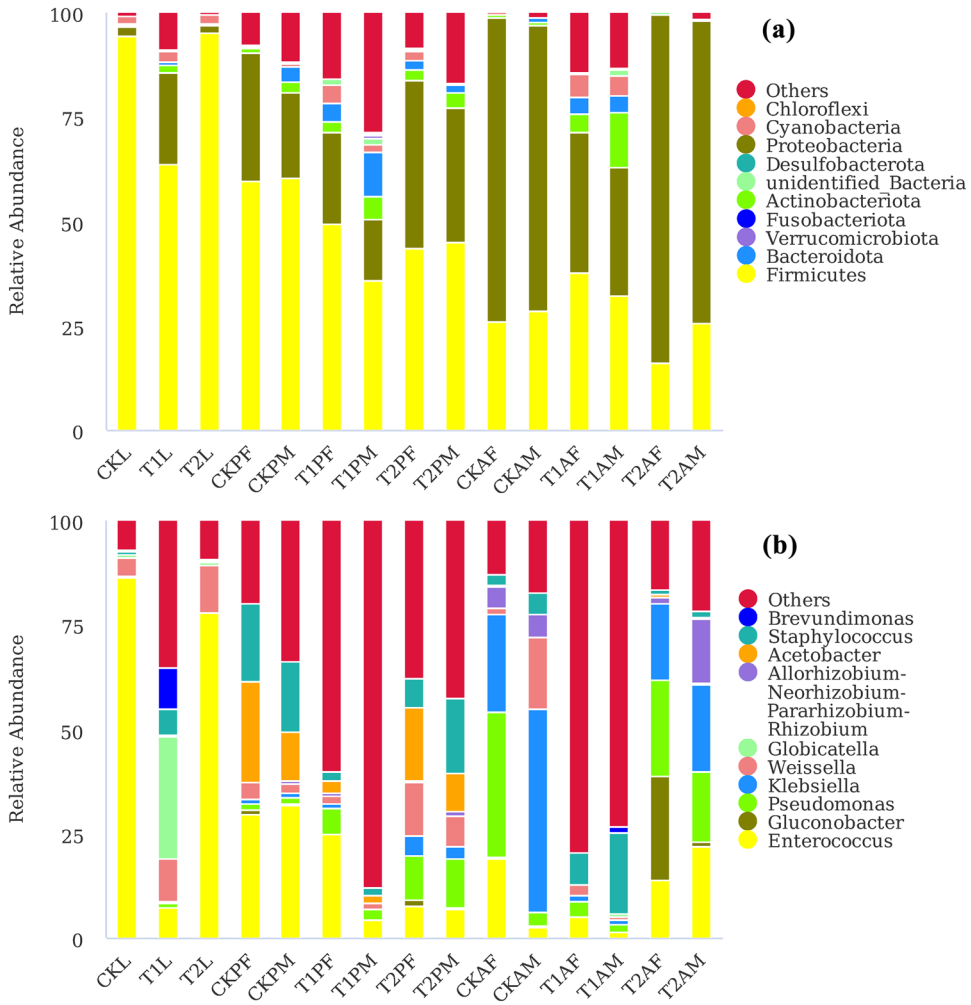


Fig. 3 Taxonomic assignment of bacterial OTUs in different samples of *S. frugiperda*. **a** Abundance at the phylum level (top 10) (see Table S4 for the complete data); **b** Abundance at the genus level (top 10) (see Table S8 for the complete data). Differences between samples for each of the top 10 phyla or genera have been presented in Tables S4 and S8

genera in T2 females, while *Enterococcus* (21.99%), *Klebsiella* (20.60%), *Pseudomonas* (16.93%) and *Allorhizobium* (15.62%) were dominant genera in T2 males.

Moreover, LDA analysis also demonstrated obvious difference on biomarks either between developmental stages or between different treatments (Fig. S3).

Effect of antibiotics feeding on development

Antibiotic treatments significantly affected larval (ANOVA: $F_{2,357} = 16.8$, $P < 0.0001$), pupal (ANOVA: $F_{2,297} = 41.8$, $P < 0.0001$ for female; $F_{2,297} = 36.8$, $P < 0.0001$ for male) and adult (ANOVA: $F_{2,177} = 51.8$, $P < 0.0001$ for female; $F_{2,177} = 9.52$, $P < 0.0001$ for male) body weight (Table 2). Post hoc analysis showed T1 had significantly lower larval/pupal/adult body weight than CK ($P < 0.05$). T2 also showed significantly lower body weight on larvae, male pupae and female adults than CK ($P < 0.05$), but not on female pupae and male adults ($P > 0.05$) (Table 2). Significant differences were also found among offspring of antibiotics-treated and control insects on larval (ANOVA: $F_{2,357} = 13.34$, $P < 0.0001$), pupal (ANOVA: $F_{2,297} = 56.46$, $P < 0.0001$ for female; $F_{2,297} = 75.28$, $P < 0.0001$ for male) and adult (ANOVA: $F_{2,177} = 45.75$, $P < 0.0001$ for female; $F_{2,177} = 19.08$, $P < 0.0001$ for male) body weight. Post hoc analysis showed that the offspring of T1 (T1-F1) had significantly lower larval/pupal/adult body weight than the offspring of control (CK-F1) ($P < 0.05$) whereas male pupae of T2-F1 only showed significantly lower body weight than that of CK-F1 ($P < 0.05$) (Table 2).

Antibiotic treatments also significantly affected the developmental period of larvae (K-W: $\chi^2 = 351.947$, $P < 0.0001$) and female pupae (K-W: $\chi^2 = 15.236$, $P < 0.0001$) (Table 2). Post hoc analysis showed T1 had the longest larval period ($P < 0.05$), followed by that of T2 ($P < 0.05$) and CK had the shortest larval period ($P < 0.05$). T2 had the longest female pupal period, which was significantly different to CK ($P < 0.05$) but not to T1 ($P > 0.05$), and no significant difference was found between CK and T1 ($P > 0.05$). Antibiotics treatment did not show significant effect on the developmental period of male pupae (K-W: $\chi^2 = 3.606$, $P = 0.165$; Table 2). The offspring showed significant differences in larval (K-W: $\chi^2 = 39.208$, $P < 0.0001$) and female pupal (ANOVA: $F_{2,297} = 7.97$, $P < 0.0001$) developmental period. Post hoc analysis showed that T1-F1 had significantly longer larval period than CK-F1 ($P < 0.05$) (Table 2). T1-F1 also showed the longest female pupal period, which was significantly longer than T2-F1 ($P < 0.05$) but not CK-F1 ($P > 0.05$) (Table 2). The offspring did not show significant differences in male pupal period (ANOVA: $F_{2,297} = 1.98$, $P = 0.14$; Table 2).

Antibiotic treatments showed significant effect on pupation rate (ANOVA: $F_{2,6} = 18.35$, $P = 0.003$; Table 2),

Table 2 Effect of antibiotics on the development of *S. frugiperda**

Treat	Larvae			Pupae				Adults		Survival rate (%)
	Body weight (mg)	Developmental period (d)	Pupation rate (%)	Body weight (mg)		Developmental period (d)		Eclosion rate (%)	Body weight (mg)	
				Female	Male	Female	Male		Female	Male	
CK	527.80 ± 5.09a	14.78 ± 0.03c	96.67 ± 0.48a	210.04 ± 2.59a	217.87 ± 2.45a	7.54 ± 0.05b	8.70 ± 0.05a	95.68 ± 0.52a	130.46 ± 2.99a	129.49 ± 3.74a	92.50 ± 0.96a
T1	491.20 ± 5.69b	16.08 ± 0.04a	90.83 ± 0.83b	183.17 ± 2.39b	188.62 ± 2.27c	7.67 ± 0.05ab	8.57 ± 0.05a	88.37 ± 2.22b	95.96 ± 2.31c	112.08 ± 2.14b	80.28 ± 2.37b
T2	490.14 ± 4.88b	15.83 ± 0.03b	95.56 ± 0.74a	208.37 ± 1.96a	200.40 ± 2.55b	7.80 ± 0.04a	8.67 ± 0.05a	91.27 ± 0.94ab	108.65 ± 1.83b	129.64 ± 3.68a	87.22 ± 1.55ab
CK-F1	535.53 ± 6.08a	13.19 ± 0.06b	96.67 ± 0.48a	202.59 ± 2.32a	211.82 ± 2.05a	7.54 ± 0.05ab	8.70 ± 0.05a	95.40 ± 1.15a	132.94 ± 3.46a	133.02 ± 3.08a	92.22 ± 1.21a
T1-F1	498.89 ± 5.73b	13.63 ± 0.10a	96.39 ± 1.55a	175.50 ± 2.26b	180.62 ± 1.99c	7.71 ± 0.06a	8.67 ± 0.06a	92.75 ± 1.38a	101.15 ± 1.85b	110.36 ± 1.99b	89.44 ± 2.74a
T2-F1	536.44 ± 5.78a	13.02 ± 0.07b	95.83 ± 0.48a	204.51 ± 1.86a	205.05 ± 0.16b	7.44 ± 0.06b	8.50 ± 0.05a	96.53 ± 1.31a	132.88 ± 2.49a	125.90 ± 2.76a	92.50 ± 0.96a

*Within each parameter (each column), values with different letters are significantly different ($P < 0.05$)

eclosion rate (ANOVA: $F_{2,6} = 8.22$, $P = 0.019$) and survival rate (ANOVA: $F_{2,6} = 13.92$, $P = 0.006$) (Table 2). Post hoc analysis showed that T1 had significantly lower pupation rate, eclosion rate and survival rate than those of CK ($P < 0.05$); T2 also showed lower value on these parameters but not significantly different to CK ($P > 0.05$) (Table 2). The offspring showed similar effect patterns, but were not statistically significant (ANOVA: $F_{2,6} = 0.25$, $P = 0.785$ for pupation rate; $F_{2,6} = 2.35$, $P = 0.176$ for eclosion rate; and $F_{2,6} = 0.82$, $P = 0.485$ for survival rate; Table 2).

Effect of antibiotics feeding on flight capacity

Antibiotic treatments negatively affected female and male flight capacity (Table 3a, b). Post hoc analysis showed that T1 treatment had stronger effects on female and male flight capacity than T2 treatment (Table 3a, b; Fig. 4a–c). The male offspring of T1 also showed significantly lower flight capacity than controls, whereas the male offspring of T2 did not show significant difference to controls (Table 3d; Fig. 4d–f). The female offspring of T1 and T2 also showed lower flight capacity than controls, but was not statistically significant (Table 3c; Fig. 4d–f).

Effect of antibiotics feeding on reproduction

Dissecting of 0-, 3- and 5-d-old virgin female adults from different treatments showed that antibiotics treatment negatively affected the development of ovary and eggs (Fig. 5a–i). In the control group, ovary/eggs development progressed rapidly from 0 to 5 days after eclosion, whereas the ovary/eggs developmental progress was relatively slow or even abnormal (such as Fig. 5d–f) in the treated groups. The offspring of T1 still showed slow ovary/eggs development pattern, which was similar to T1, whereas T2 offspring showed normal ovary/eggs development pattern, i.e., similar to controls (Fig. 5j–r).

Antibiotic-treated females laid fewer eggs daily than those of controls (Fig. 6a–c). The offspring of T1 also laid fewer eggs daily than those of controls (Fig. 6d, e), whereas T2 offspring laid similar number of eggs daily compared to those of controls (Fig. 6d, f).

Antibiotic treatments had a significant negative effect on female reproductive fitness (Table 3e). Post hoc analysis showed that T1 and T2 females laid significantly fewer eggs and larvae than controls ($P < 0.05$; Fig. 7a). The egg-hatching rate of T1 was also significantly lower than those of T2 and controls ($P < 0.05$), whereas that between T2 and controls showed no significant difference ($P > 0.05$) (Fig. 7b). Antibiotic treatments did not show significant effect on female longevity (ANOVA: $F_{2,57} = 2.7$, $P = 0.076$).

Table 3 Effect of antibiotics on the flight capacity and reproduction of *S. frugiperda**

Model	Description	Parameter	*d.f*	*F*	*P*
(a)	Flight capacity of females treated with antibiotics	Whole model	6.202	5.028	=0.003
		Flight distance	2.102	6.333	=0.003
		Flight duration	2.102	2.950	=0.057
		Flight speed	2.102	6.198	=0.003
(b)	Flight capacity of males treated with antibiotics	Whole model	6.202	6.303	=0.001
		Flight distance	2.102	9.536	<0.0001
		Flight duration	2.102	6.476	=0.002
		Flight speed	2.102	3.879	=0.024
(c)	Flight capacity of female offspring	Whole model	6.202	1.439	=0.236
(d)	Flight capacity of male offspring	Whole model	6.202	3.611	=0.016
		Flight distance	2.102	3.267	=0.042
		Flight duration	2.102	3.753	=0.027
		Flight speed	2.102	1.293	=0.297
(e)	Reproduction of insects treated with antibiotics	Whole model	6.112	13.225	<0.0001
		No. of eggs laid	2.57	16.390	<0.0001
		No. of larvae	2.57	18.385	<0.0001
		Egg-hatching rate	2.57	7.381	=0.001
(f)	Reproduction of offspring	Whole model	6.112	11.496	<0.0001
		No. of eggs laid	2.57	15.542	<0.0001
		No. of larvae	2.57	16.636	<0.0001
		Egg-hatching rate	2.57	5.673	=0.006

*Analysed using a multivariate analysis of variance (MANOVA)

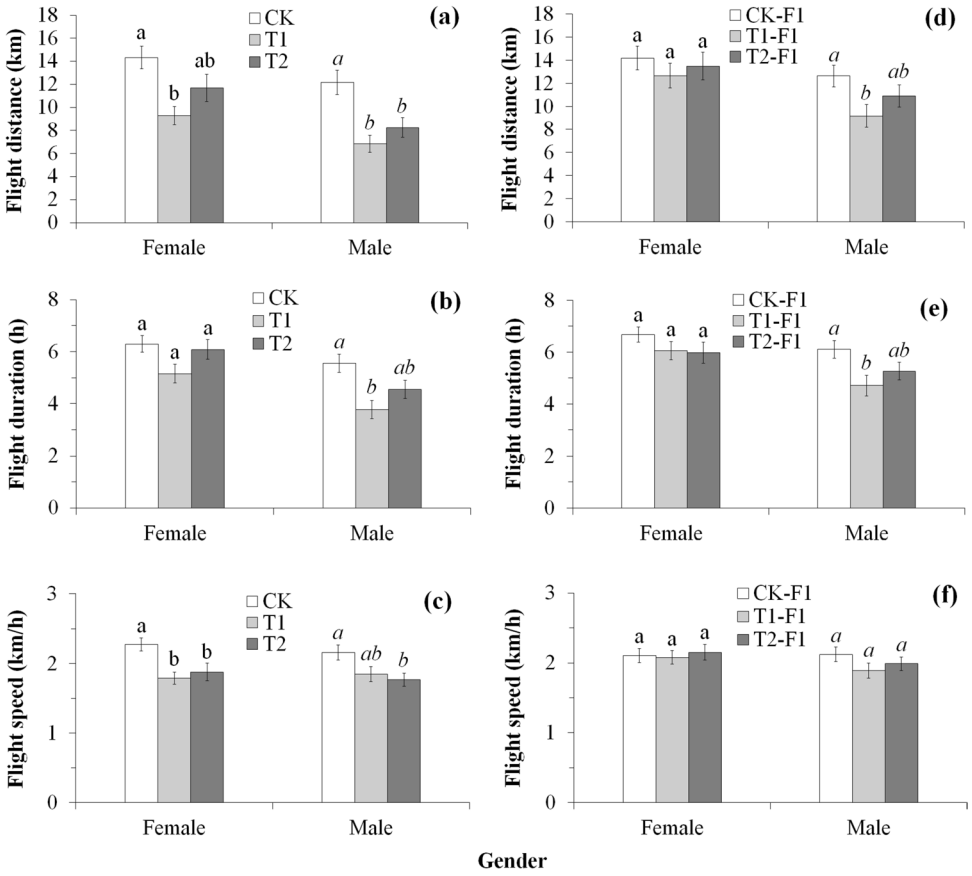


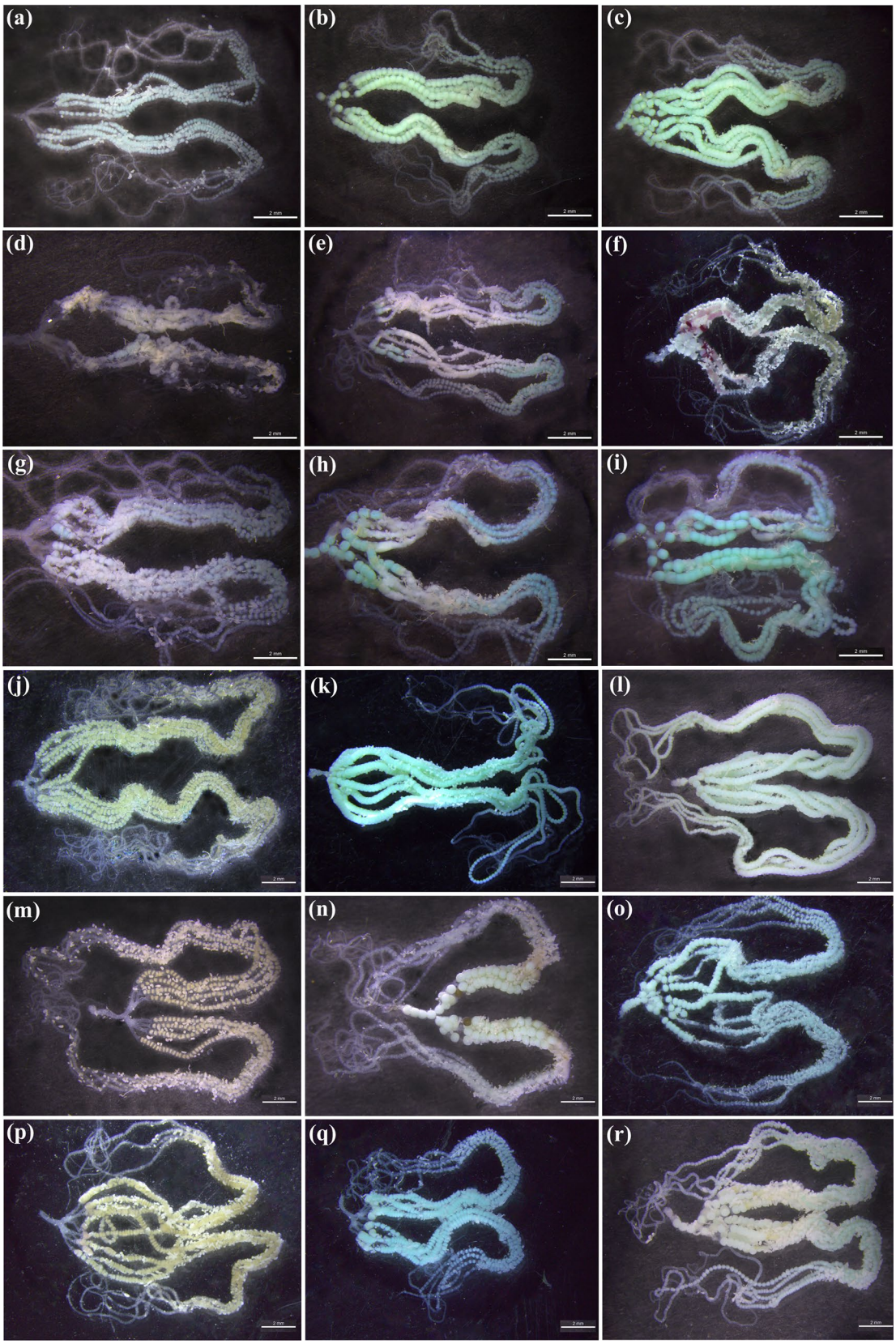
Fig. 4 Effect of antibiotic treatments on flight capacity of *S. frugiperda*. **a**, **b** and **c** indicate flight distance, flight duration and flight speed of treated insects (T1, T2 and CK), respectively; **d**, **e** and **f** indicate flight distance, flight duration and flight speed of the offspring (T1-F1, T2-F1 and CK-F1) from treated insects, respectively. Within differences of females or males in each sub-figure (regular letters were used for females and italic used for males), bars with different letters are significantly different ($P < 0.05$)

The offspring of T1 also showed significant lower reproductive fitness than controls (Table 3f; Fig. 7d, e), whereas T2 offspring did not show significant difference to controls (Fig. 7d, e). Also female longevity between offspring of antibiotic-treated and control insects did not show a significant difference (ANOVA: $F_{2,57} = 1.35$, $P = 0.267$) (Fig. 7f).

Discussion

Previous studies have observed differences in the gut microbial community of *S. frugiperda* among populations of different host plants (Lv et al. 2021), and among different geographic populations and between larvae and adults (Gichuhi et al. 2020). Results of the present study showed that the number of OTUs was low in larvae (195–280) and adults (160–359), but high in pupae (491–916) in untreated or antibiotic-treated insects (Table 1); alpha diversity indices also indicated a variation in bacterial diversity among different stages accordingly (Table 1). Previous studies have reported on the reduction in gut microbiota in the pupal stage (compared to larvae), such as in the carrion beetle, *Nicrophorus vespilloides* (Wang and Rozen 2017), the honeybee, *Apis dorsata* (Saraithong et al. 2017), the borer, *Hypothenemus hampei* (Santiago Mejia-Alvarado et al. 2021) and the moth, *P. xylostella* (Lin et al. 2015). On the other hand, a few studies have also reported the pupal stage as that which had the highest diversity in gut microbiota, such as in the wood borer, *Agrilus mali* (Zhang et al. 2018) and the subcortical beetle, *Agrilus planipennis* (Vasanthakumar et al. 2008). Holometabolous insects experience dramatic metamorphosis from larva to adult, which involves a complete remodeling of internal and external anatomy during pupation and pupal development (Grimaldi et al. 2005). Compared with larvae and adults, the pupal gut may undergo morphological changes and decreased metabolic activity, which may impact the composition and diversity of the gut microbial community (Morales-Jimenez et al. 2012). Possibly, the pupae may lose some core gut bacteria during the pupal development, allowing a greater number of transient or non-specific bacteria to be present in the sequenced data (Zhang et al. 2018).

The bacterial OTU richness and diversity (indicated by the Shannon, Simpson and Chao1 indexes) showed differences between control and antibiotic treatments (Table 1). Beta diversity analysis based on the Bray–Curtis distance further showed significant difference between CK and T1 and between T1 and T2 ($P < 0.05$), whereas that between CK and T2 was not significant ($P > 0.05$) (Fig. 2b; Table S3). This result suggests that the effect of antibiotic on microbial community in *S. frugiperda* was dose-dependent, as T2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◂**Fig. 5** Effect of antibiotic treatments on ovary and egg development in *S. frugiperda*. **a**, **b** and **c** indicate 0-, 3- and 5-d-old CK female ovary, respectively; **d**, **e** and **f** indicate 0-, 3- and 5-d-old T1 female ovary, respectively; **g**, **h** and **i** indicate 0-, 3- and 5-d-old T2 female ovary, respectively; **j**, **k** and **l** indicate 0-, 3- and 5-d-old CK-F1 female ovary, respectively; **m**, **n** and **o** indicate 0-, 3- and 5-d-old T1-F1 female ovary, respectively; and **p**, **q** and **r** indicate 0-, 3- and 5-d-old T2-F1 female ovary, respectively. Scale = 2 mm

treatment only lasted 8 days during the larval stage, while T1 treatment covered the whole larval and adult stages. A further fitness assay also confirmed that antibiotic treatment dramatically affected the development, flight capacity and reproductive fitness of *S. frugiperda*, with treated insects having longer developmental period, but lower body weight, survival rate, flight capacity and fecundity than those of controls. Developmental parameters (including body weight, developmental period, and pupation, eclosion and survival rate; Table 2) and flight capacity (Fig. 4) also showed dose-dependent effects, with T2 showing a lower effects than T1 in most cases; this was exhibited by the survival rate, which was 92.50% for CK, followed by T2 as 87.22%, and then T1 as 80.28% (Table 2). Fecundity tests showed that T1 and T2 females laid a significantly lower number of eggs and offspring than those of controls. Eggs of T1 females even showed significantly lower hatching rate than controls. Moreover, the effects of antibiotic treatments on reproduction in *S. frugiperda* would have been underestimated if we considered the survival rate. To exclude the possible side-effect of antibiotic treatment on *S. frugiperda*, we further evaluated the fitness of the offspring from antibiotics-treated insects. The result also showed that T1 offspring had significantly longer larval developmental period, lower larval/pupal/adult body weight, and lower reproductive output than those of controls (Table 3; Fig. 6d–f; Fig. 7d–f). Moreover, the flight capacity of T1 offspring was also lower than controls, which was significant in males but not in females. T2 offspring did not show significant difference in fitness compared to controls in most cases, which may be also due to dose-dependent effects.

Gut microbes are important for providing nutrition, protection from parasites and pathogens, modulation of immune responses, and communications with environment in hosts (Jang and Kikuchi 2020; Engel and Moran 2013). A dynamic homeostasis between the host and symbiotic microbes is essential for animals (Mason et al. 2011), whereas a dysbiosis gut can affect the metabolic process (Sartor 2008; Hamdi et al. 2011). For example, *S. frugiperda* larvae fed on artificial diet containing antibiotics, showed dramatic changes in the composition and diversity of gut bacterial community, where Firmicutes was decreased, and the richness of *Enterococcus* and *Weissella* was largely reduced. Further transcriptome analysis showed that antibiotics-induced dysbiosis affected many biological processes in *S. frugiperda*, such as metabolism and energy production (Chen et al. 2021). In the present study, we also found some similar changes between control and antibiotics-treated groups, such as the decrease in Firmicutes (Fig. 3a) and *Enterococcus* abundance in larvae (Fig. 3b). These results may partially explain the negative effect of antibiotic treatment on the development and reproduction in *S. frugiperda*.

Gut *Enterococcus* in the Lepidoptera play a protective role against insect pathogens (Voirol et al. 2018). For instance, *Enterococcus mundtii* was reported as the highly abundant gut bacterium in the cotton leafworm, *S. littoralis*, which produce antimicrobial compounds against G^+ pathogens (such as *Listeria*), but harmless to other resident bacteria (Voirol et al. 2018; Shao et al. 2017). Also, *E. faecalis* found in the gypsy moth protect the host against pathogenic toxins that are activated in alkaline conditions (such as Bt) by acidifying the local environment (Broderick et al. 2004). The reduction of *Enterococcus* in antibiotic-treated individuals (larval and pupal stages) may render them more susceptible to pathogens. However, feeding adults of Oriental fruit fly with *E. faecalis* decreased their fecundity (Akami et al. 2019). A recent study on the pumpkin fruit fly also suggested that *Enterococcus* had a negative impact on fecundity (Noman et al. 2021).

Our results also showed an obvious reduction in the abundance of *Klebsiella*, in the adult stage of *S. frugiperda*, especially in T1-treated males and females (Fig. 3b). Similarly, the fecundity of females of the Chinese citrus fly, *Bactrocera minax*, was reduced after bacteria were removed by feeding of antibiotics, while it increased when the flies were fed with a normal diet supplemented with three bacterial species (*Klebsiella pneumonia*, *Citrobacter braakii* and *Pantoea dispersa*) (Rashid et al. 2018).

Our study also revealed that *Weissella* (during larval stage) and *Pseudomonas* (mostly during pupal and adult stages) abundance was higher in treated individuals. Some *Pseudomonas* species are able to degrade lignocellulose (Yang et al. 2007) and some *Weissella* species can produce bacteriocins, organic acids, and adhesion inhibitors to inhibit the growth of other bacteria (Masuda et al. 2012; Woraprayote et al. 2015). However, some *Pseudomonas* and *Weissella* strains are insect or animal pathogens or opportunistic pathogens (Fusco et al. 2015). Opportunistic pathogenic strains usually exert no negative effects on insect health; however, they may switch from a gut symbiont to a systemic pathogen under certain conditions, such as underlie Bt killing mechanism, which adversely affects insect development (Mason et al. 2011; Caccia et al. 2016).

These results suggest that some bacteria, such as *Enterococcus* spp and *Pseudomonas* spp may have opposite function (beneficial or detrimental) under different condition or in different hosts, which may be due to coevolution between them and the hosts (Voirol et al. 2018; Groussin et al. 2020).

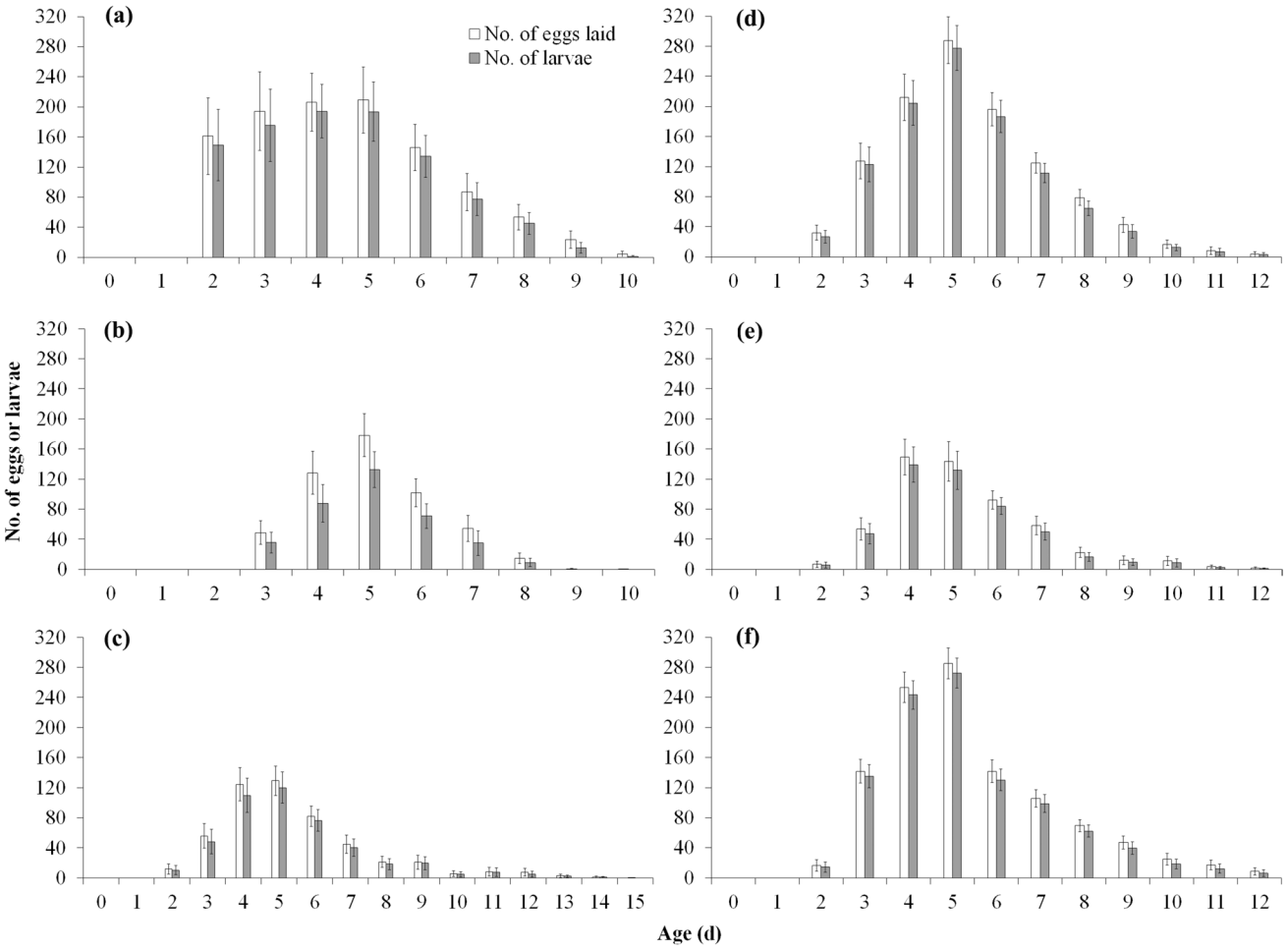

Fig. 6 Effect of antibiotic treatments on daily oviposition of CK (**a**), T1 (**b**), T2 (**c**), CK-F1 (**d**), T1-F1 (**e**) and T2-F1 (**f**) in *S. frugiperda*

It may also be due to a dysbiosis of the homeostasis between the host and gut microbes (Hamdi et al. 2011; Mason et al. 2011; Chen et al. 2021).

Gut microbes have also been reported to have beneficial or detrimental effects on reproduction in insects (Andongma et al. 2018; Akami et al. 2019). However, the underlying mechanisms are still poorly understood. In the present study, dissection of virgin female adults revealed that antibiotic treatment negatively affected the development of ovary and eggs in *S. frugiperda* (Fig. 5). A recent study in the pumpkin fruit fly also found that antibiotic treatment inhibited ovary development and oviposition (Noman et al. 2021). Antibiotics-induced dysbiosis of gut microbes may affect many biological processes in *S. frugiperda*, such as metabolism and energy production (Chen et al. 2021). Moreover, most lepidopterans lack specific degrading enzyme encoding genes, such as for cellulase and pectinases (Voirol et al. 2018), and may mainly rely on their microbiome for such degradations. Therefore, the antibiotics-induced dysbiosis of gut microbes in *S. frugiperda* may result in nutritional stress and/or pathogen susceptibility, which then negatively affect its development, survival and fecundity. However, the resultant nutritional stress may also be due to other factors, as we fed all test insects with high quality diets.

Wolbachia has been reported as resident in most lepidopteran species (Ahmed et al. 2015), which may have positive effect on development and reproduction (Moriyama et al. 2015; Voirol et al. 2018). However, we did not find *Wolbachia* infection in all samples of *S. frugiperda*. Therefore, the effect of *Wolbachia* on the reduction in development and reproduction in antibiotic-treated *S. frugiperda* was minimal. Moreover, *Serratia*, *Clostridium* and *Enterobacter*, strains identified as opportunistic pathogen in insect (Mason et al. 2011; Broderick et al. 2006; Caccia et al. 2016), showed high abundance in *S. frugiperda* (Table S8). Their presence may thus enhance Bt insecticidal activity (Caccia et al. 2016). A recent study in *S. frugiperda* showed that bacteria isolated from field-collected larvae grew better and showed potential to metabolize more insecticides than that from laboratory-selected resistant strains (Gomes et al. 2020). These findings

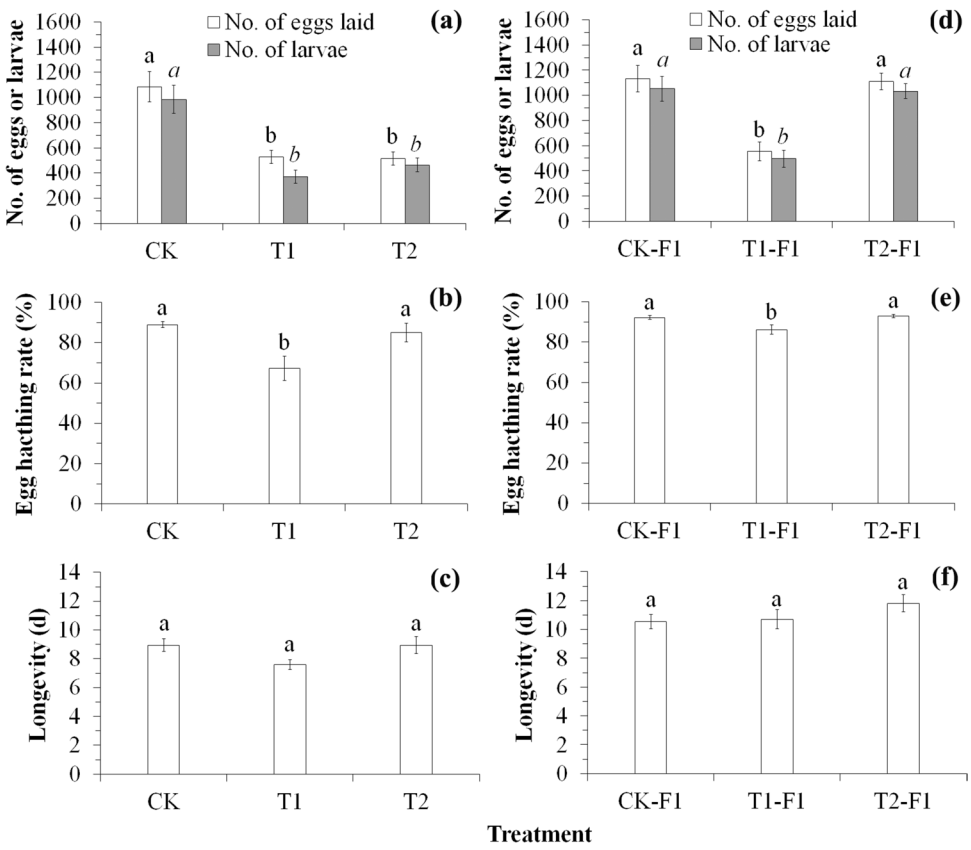

Fig. 7 Effect of antibiotic treatments on the reproductive fitness of *S. frugiperda*. **a** and **d** indicate number of eggs laid and larvae of treated insects and their untreated offspring, respectively; **b** and **e** indicate egg-hatching rate of treated insects and their untreated offspring, respectively; and **c** and **f** indicate female longevity of treated insects and their untreated offspring, respectively. Within differences of each parameter in each sub-figure (regular and italic letters were used in sub-figures which has two parameters), bars with different letters are significantly different ($P < 0.05$)

further suggest that the homeostasis between the host and gut microbes is essential for insects, whereas dysbiosis gut can affect the host's survival and reproduction, which have important implications for applied control strategies.

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Author contributions L-YZ and HY collected and reared the insects; L-YZ, YF, D-YF, JX and SY designed the study; YF, L-YZ, Q-YZ, D-YF and HY performed the experiments; YF, L-YZ, HY, D-YF, and JX analyzed the data; L-YZ, YF, JX and SY wrote the paper. All authors read and approved the final manuscript.

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Data availability The raw reads from 16S rDNA gene sequencing were deposited into the NCBI (https://www.ncbi.nlm.nih.gov/) Sequence Read Archive (SRA) database, the login number is PRJNA803874. Other data generated or analyzed during this study are included in this article and its supplementary material.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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